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Effect of dietary lipid on growth performance, body composition, plasma biochemical parameters and liver fatty acids content of juvenile yellow drum *Nibea albiflora*

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ABSTRACT

A feeding trial was conducted to determine the dietary lipid requirement and its effects on body composition, plasma biochemical parameters and liver fatty acids content in juvenile yellow drum *Nibea albiflora*. Six animal groups (initial weights, 17.7 ± 0.20 g) were fed isonitrogenous diets formulated with increasing lipid levels (52, 70, 94, 111, 129 and 153 g kg^{-1}, labeled as L50, L70, L90, L110, L130, L150, respectively) using menhaden oil, twice daily to apparent satiation, for 8 weeks. The results showed that the weight gain and specific growth rate (SGR) of fish fed L130 and L150 lipid diets were significantly higher than those of the animals on the L50 lipid diet. The feed conversion rate (FCR) of fish fed the L130 lipid diet was significantly lower compared with the values obtained for the other groups. Hepatosomatic index (HSI) of fish fed the L90 lipid diet was significantly higher than that of animals on L150 lipid. Whole body and muscle lipid contents increased with increasing dietary lipid level, and the dietary fatty acid profile was reflected in liver tissue. Liver eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents in fish fed with the L150 diet were significantly higher compared with the values recorded in the other groups. Total highly-unsaturated fatty acid (HUFA) content in liver showed an increasing trend, whereas total saturated fatty acid (SFA) and mono-unsaturated fatty acid (MUFA) contents in liver tended to decrease with increasing dietary lipid levels. The plasma triglyceride and cholesterol contents of juvenile *N. albiflora* increased with the increasing dietary lipid level. Analysis by the broken-line model of percent weight gain indicated the optimal dietary lipid level in juvenile *N. albiflora* to be 120 g kg^{-1} of the diet.

1. Introduction

Dietary lipid provides essential fatty acids, phospholipids, sterols and fat-soluble vitamins necessary for proper functioning of physiological processes as well as maintenance of biological structure and cell membranes function (Sargent et al., 1989; Ghanawi et al., 2011). It also serves as an energy source for protein sparing (De Silva and Anderson, 1995; Mishra and Samantaray, 2004). A clear dose-response effect of dietary crude lipids was observed on growth of the Gulf corvina, *Cynoscion othonopterus* (Gonzalez-Felix et al., 2015) and *Huso huso* (Ahmadi Fackjouri et al., 2011). Within certain limits, increased dietary lipid levels can also improve diet utilization (Du et al., 2005). However, excess dietary lipid supplement may cause growth retardation (Gonzalez-Felix et al., 2015), and even confer extreme metabolic burden to the liver (Jin et al., 2013), resulting in excessive fat deposition (Martins et al., 2007; Song et al., 2009; Ghanawi et al., 2011). Therefore, it is important to formulate diets with proper lipid levels to meet the energy and fatty acid requirements for fish (Lopez et al., 2009). Studies have demonstrated marine species need n-3 highly unsaturated fatty acids (HUFA) from dietary lipid to maintain the normal body fatty acid composition and physiological functions (Glencross, 2009; Zuo et al., 2012) due to marine teleosts lack the ability to synthesize their own essential HUFA from the 18 carbon unit precursors (Zakeri et al., 2011). Inadequate contents of n-3 HUFA give rise to several alterations such as poor feeding, immune-deficiency (Izquierdo, 1996) and a delay in the functional development of brain and vision (Benitez-Santana et al., 2007). Therefore, both dietary

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lipid quality and quantity are significant for growth and development of fish (Mishra and Samantaray, 2004).

The yellow drum (*Nibea albiflora*, Sciaenidae), is an emerging commercially important marine species distributed along the coasts of East Asia (Han et al., 2008). Currently, information is available for *N. albiflora* regarding see production (Sun et al., 2005), genetic population structure (Han et al., 2008), genetic diversity (Xu et al., 2012) and sperm cryopreservation (Dai et al., 2012). However, to our knowledge, there is little information available concerning the nutritional requirements of juvenile yellow drum. Lu et al. (2015) reported that the optimal protein requirement of juvenile *N. albiflora* was 55.39%. The use of formulated feeds for growing *N. albiflora* to market size would be more practical and efficient in terms of labor costs, compared with the present practice of using discarded fish as the rearing diet. This study aimed to determine the dietary lipid requirement, and its effects on body composition, plasma biochemical parameters and liver fatty acids content in juvenile *N. albiflora*.

2. Materials and methods

2.1. Experimental diets

Six experimental diets were formulated to contain graded levels of dietary lipid (52, 70, 94, 111, 129 and 153 g kg^{-1}, labeled as L50, L70, L90, L110, L130, L150, respectively), prepared with menhaden oil as the main lipid source (Table 1). Fish, soybean, and wheat gluten meals were used as protein sources. All ingredients, purchased from Ningbo Tech-Bank Feed Co. Ltd, were ground into fine powder with particle size of less than 60 meshes. The micro-components were then mixed by progressive enlargement. Lipids and distilled water were then added to the premixed dry ingredients and mixed to homogeneity in a Hobart type mixer. Cold-extruded pellets were produced, and pellet strands broken into uniform pellet sizes (3.0 mm and 5.0 mm diameter, respectively) with a granulating machine (G-250, Machine factory of South China University of Technology, Guangzhou, China), steamed for 30 min at 90 °C, and air-dried to approximately 10% moisture; the resulting single pellets were sealed in vacuum-packed bags and stored at −20 °C until use in the feeding trial.

2.2. Animals and feeding

Juvenile *Nibea albiflora* individuals from our fish farm (XiShan Technology Island, Zhoushan of Zhejiang Province, China) were acclimatized, consuming a commercial diet for 2 weeks prior to the feeding trial. At the beginning of the experiment, juvenile *N. albiflora* (initial weight 17.7 ± 0.20 g, mean ± SD) of similar sizes were transferred into 18 cylindrical fiberglass tanks (500-L), with 20 individuals per tank. Diets were randomly assigned to triplicate groups of fish. Each tank was provided with a continuous flow of water (2 L min^{-1}) and uninterrupted aeration through air stones to maintain dissolved oxygen levels at or near saturation. All fish groups were fed twice daily at 08:00 h and 17:00 h to satiation. During the experimental period, the following conditions were applied: temperature, 26–28 °C; pH, 7.6–7.8; salinity, 27.0 ± 1.00 g L^{-1}; unionized ammonia nitrogen <0.05 mg L^{-1}; dissolved oxygen >6.0 mg L^{-1}. Each tank was cleaned biweekly, when the fish were removed and weighed as a group.

2.3. Sample collection and chemical analysis

At the end of the 8-week feeding trial, *N. albiflora* in each tank were individually weighed afterfasting for 24 h. Body lengths, viscera and liver weights were measured to calculate viscerosomatic index (VSI), hepatosomatic index (HSI) and condition factor(CF). Five representative fish from each tank were anesthetized with tricaine methanesulfonate (MS-222; Sigma, 40 mg L^{-1}), and approximately 0.5 mL of blood was collected from the caudal vasculature using a 1-ml syringe with 27-gauge needle. Plasma was separated after centrifuging at 4000 g for 10 min at 4 °C using a high-speed refrigerated centrifuge (Eppendorf 5810R, Germany) and stored at −80 °C for further analyses. Livers and back muscles dissected from five fish were stored at −20 °C for fatty acid and proximate analysis, respectively. Additional five fish were randomly captured from each tank for whole body analysis.

Crude protein, lipid, moisture and ash content in diets, whole body of fish and back muscle in fish were determined according to the methods of the Association of Official Analytical Chemists (AOAC, 1995). Moisture content was determined by oven drying at 105 °C to a constant weight. Crude protein content (N × 6.25) was determined via the Kjeldahl method, following an acid digestion with an Auto-digester (KjelFlex K-360, BUCHI, Switzerland). Crude lipid content was determined by the ether extraction method, using a Soxtec System HT (Soxtec 2055, FOSS Tecator, Sweden). Ash content was determined using a muffle furnace, maintained at 550 °C for 8 h.

The plasma biochemical parameters were analysed using an automatic biochemistry analyzer (Beckman DXC800, USA).

Fatty acid methyl esters (FAMEs) in the liver tissue samples were prepared according to Kirsch et al. (1998), with 7% boron trifluoride in methanol and a temperature of 100 °C for 1 h. The FAMEs were separated with a gas chromatograph, equipped with a flame-ionization detector (Agilent 6890 GC system, Agilent, USA) on a capillary column (60 m × 0.25 mm; 0.25 μm). The fatty acids were identified by comparing their retention times with those of known standards and are expressed as a percentage of the total FAME content.

2.4. Calculations and statistical analysis

The following parameters were used for evaluating growth performance:

$$\text{Survival} = \text{Nt} \times 100/\text{N0}$$

$$\text{Weightgain} = 100 \times (\text{Wt} - \text{W0})/\text{W0}$$

$$\text{Specificgrowthrate(SGR, \%day}^{-1}) = 100 \times (\text{LnWt} - \text{LnW0})/\text{t}$$

$$\text{Feedconversionrate(FCR)} = [\text{feedconsumed(g, dryweight)}/\text{weightgain(g)}] \times 100$$

$$\text{Proteinefficiency(PER)} = \text{weightgain(g)}/\text{proteinintake(g)}$$

$$\text{Conditionfactor(CF, \%)} = 100 \times (\text{bodyweight, g})/(\text{bodylength, cm})^3$$

$$\text{Hepatosomaticindex(HSI, \%)} = 100 \times (\text{liverweight, g}/\text{wholebodyweight, g})$$

$$\text{Viscerosomaticindex(VSI, \%)} = 100 \times (\text{viscera weight, g})/(\text{wholebodyweight, g})$$

Table 1
Composition of experimental diets (g kg^{-1} dry weight).

Ingredients	Experimental diets					
	L50	L70	L90	L110	L130	L150
Fish meal	370	370	370	370	370	370
Soybean meal	200	200	200	200	200	200
Wheat protein powder	120	120	120	120	120	120
Fish oil	5	25	45	65	85	105
Dextrin	253.5	209.6	165.7	121.8	77.9	33.9
Soybean lecithin	15	15	15	15	15	15
[a]Vitamin premix	5	5	5	5	5	5
[b]Mineral premix	3	3	3	3	3	3
Choline chloride	3	3	3	3	3	3
$Ca(H_2PO_4)_2$	5	5	5	5	5	5
Antioxidant	0.5	0.5	0.5	0.5	0.5	0.5
[c]CMC	20	20	20	20	20	20
Cellulose	0	23.9	47.8	71.7	95.6	119.6
Proximate composition						
Crude protein	457	453	449	456	451	448
Crude lipid	52	70	94	111	129	153
Ash	81.1	81.9	79.7	80.6	82.1	82.1

[a] Vitamin premix contained (mg kg^{-1} diet): thiamine, 25; riboflavin, 36.7; vitamin A, 32; vitamin E, 120; vitamin D3, 5; menadione, 5.1; pyridoxine HCl, 20; cyanocobalamin, 0.1; biotin, 1.2; calcium pantothenate, 60; folic acid, 20; niacin, 200; inositol, 792; vitamin C, 2000. All ingredients were diluted with cellulose to 1 g.
[b] Mineral premix contained (mg kg^{-1} diet): magnesium sulfate, 1826; ferrous sulfate, 119; zinc sulfate, 76; manganese sulfate, 44; cobalt chloride, 2; potassium iodide, 0.8; copper sulfate, 1; sodium chloride, 100; monopotassium phosphate, 233.2; monosodium phosphate, 137.0. All ingredients were diluted with cellulose to 1 g.
[c] CMC sodium carboxymethyl cellulose.

where, Wt and W0 were final and intial fish weights, respectively; Nt and N0 were final and initial fish number in each tank, respectively; t is the experimental duration in day.

Data are mean ± standard error (SE) and were assessed by one-way analysis of variance (ANOVA). When significant differences ($P < 0.05$) were obtained, group means were further evaluated with Turkey's multiple comparison tests. Statistical analyses were performed using SPSS 16.0 (SPSS, Inc., USA). The broken-line model was employed to assess the optimum dietary lipid level.

3. Results

The dietary lipid level affected fish growth significantly, as indicated by the growth performance, feed utilization, morphometrical parameters, body composition, and liver fatty acid profile (Tables 3–5). The weight gain and specific growth rate (SGR) of fish fed L130 and L150 lipid diets were significantly higher than those of the animals on L50 lipid diet. The feed conversion rate (FCR) of fish fed the L130 lipid diet was significantly lower compared with the values obtained for the other groups. No significant differences were observed in survival and protein efficiency rates (PER) ($P > 0.05$), which were increased before falling with increasing dietary lipid levels. Hepatosomatic index (HSI) of fish fed the L90 lipid diet was significantly higher than that of fish on L150 lipid. The highest viscera somatic index (VSI) was observed in the L50 lipid diet group. The condition factor (CF) was not significantly affected by the dietary treatments ($P > 0.05$).

Whole body lipid content increased with increasing dietary lipid levels and reached a plateau at L90 diet (Table 5) However, no significant differences were found in whole body moisture, protein and ash content among the treatments. The muscle lipid content increased significantly with dietary lipid levels, and fish fed the L150 lipid diet was significantly higher than those of fish fed the L50 and L70 lipid diets. Similarly, no significant differences were found in muscle moisture, protein, and ash content among the treatments.

The fatty acid composition of the experimental diets is presented in Table 2. The C16:0, C18:1n-9, C20:5n-3(EPA) and C22:6n-3(DHA) were the most abundant of the saturated, mono and polyunsaturated fatty acids in the experimental diets. The fatty acid profiles in the dietary were well reflected by the liver fatty acid profiles in *N. albiflora*. The main saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids in the liver of juvenile *N. albiflora* were C14:0, C16:0, C16:1, C18:1n-9, C18:2n-6 C20:5n-3, and C22:6n-3 (Table 7). Liver eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents in fish fed the L150 lipid diet were significantly higher compared with the values recorded in the other groups. Total highly-unsaturated fatty acid (HUFA) content in liver showed an increasing trend, whereas total saturated fatty acid (SFA) and mono-unsaturated fatty acid (MUFA) contents in liver tended to decrease with increasing dietary lipid levels.

The plasma triglyceride contents of juvenile *N. albiflora* fed L150 lipid diet were significantly higher compared with the values obtained for the other groups except the L130 lipid group (Table 6). The lowest plasma cholesterol contents were found in juvenile *N. albiflora* fed L50 lipid diet. No significances were observed in plasma total protein contents among all treatments. The plasma glucose contents first showed an increasing and then a slightly decreasing trend with increasing dietary lipid levels.

4. Discussion

It is important to formulate diets with proper lipid levels in order to meet the energy and fatty acid requirements for fish (Lopez et al., 2009). Previous studies demonstrated that dietary lipid level significantly enhanced growth performance of fish such as Gulf corvina *Cynoscion othonopterus* (Gonzalez-Felix et al., 2015), grass carp *Ctenopharyngodon idella* (Jin et al., 2013) and White seabass *Atractoscion nobilis* (Lopez et al., 2009). Similarly, in this study, weight gain and specific growth rate (SGR) of fish fed L130 and L150 lipid diets were significantly lower than the values obtained for the L50 lipid diet group, which may indicate a protein sparing effect of dietary lipids (Shapawi et al., 2014). The feed conversion rate (FCR) of fish fed the L130 lipid diet was significantly higher than that of fish fed the L50 lipid diet, suggesting that juvenile *Nibea albiflora* could utilize dietary lipid effectively up to a certain level (Kikuchi et al., 2009). The relationship between fish weight gain and dietary lipid level was assessed using the broken-line model, and a break point was obtained at 120 g kg^{-1} (Fig. 1), in agreement with data reported for other members of the family Sciaenidae such

Table 2
Fatty acid composition (mg g^{-1}) of the experimental diets.

Lipid level	L50	L70	L90	L110	L130	L150
C14:0	2.54	3.61	5.22	6.49	7.18	9.32
C15:0	0.35	0.49	0.67	0.82	0.81	1.10
C16:0	10.69	14.73	19.03	22.88	24.48	31.97
C16:1	2.58	3.75	5.32	6.63	7.97	9.47
C17:0	0.37	0.51	0.67	0.84	0.91	1.15
C17:1	0.22	0.34	0.50	0.63	0.76	0.93
C18:0	2.15	2.90	3.63	4.43	4.93	6.14
C181n9c	7.94	10.67	13.37	15.88	19.77	21.19
C18:2n6c	5.10	6.15	6.72	7.35	8.80	8.56
C20:0	0.47	0.81	1.14	1.46	1.83	2.28
C20:1n9	1.12	1.75	2.25	2.88	3.66	3.99
C18:3n3	1.22	1.64	1.99	2.27	2.72	2.92
C22:1n9	1.71	2.41	3.06	4.43	5.15	6.34
C20:4n6	0.46	0.59	0.72	0.90	1.07	1.09
C22:2	0.21	0.33	0.44	0.55	0.72	0.79
C20:5n3 (EPA)	4.00	6.14	8.26	10.36	12.87	14.26
DPA	0.35	0.49	0.63	0.80	0.99	1.10
C22:6n3 (DHA)	6.92	10.14	13.06	16.24	20.36	21.56
$\sum$SFA	16.78	23.32	30.64	37.12	40.51	52.37
$\sum$MUFA	13.80	19.27	24.92	31.00	37.99	42.71
$\sum$n-3	12.49	18.41	23.93	29.68	36.94	39.83
$\sum$n-3HUFA	11.27	16.77	21.94	27.40	34.22	36.91

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA saturated fatty acids; MUFA mono-unsaturated fatty acids; HUFA highly-unsaturated fatty acids.

Table 3
The weight gain, survival, specific growth rate (SGR), feed conversion rate (FCR) and protein efficiency rate (PER) of juvenile *Nibea albiflora* fed different levels of lipid diets.

Lipid level	Weight gain(%)	Survival (%)	SGR (% d^{-1})	FCR	PER
L50	253 ± 41.8^{a}	86 ± 8.54	2.42 ± 0.23^{a}	1.80 ± 0.19^{b}	1.66 ± 0.38
L70	294 ± 34.4^{ab}	81.7 ± 6.01	2.63 ± 0.17^{ab}	1.40 ± 0.29^{ab}	1.61 ± 0.30
L90	318 ± 24.1^{ab}	86.0 ± 8.54	2.75 ± 0.11^{ab}	1.28 ± 0.32^{ab}	1.69 ± 0.32
L110	339 ± 31.1^{ab}	93.7 ± 7.09	2.84 ± 0.14^{ab}	1.14 ± 0.07^{ab}	1.89 ± 0.06
L130	353 ± 31.4^{b}	96.7 ± 5.77	2.90 ± 0.13^{b}	1.00 ± 0.05^{a}	2.16 ± 0.02
L150	350 ± 14.9^{b}	81 ± 8.48	2.88 ± 0.06^{b}	1.39 ± 0.42^{ab}	1.69 ± 0.08

Data represent mean ± S.E. (n = 3). Values in the same column with different superscripts are significantly different (P < 0.05).

Table 4
The hepatosomatic index (HSI), viscera somatic index (VSI) and condition factor (CF) of juvenile *Nibea albiflora* fed different levels of lipid diets.

Lipid level	HSI (%)	VSI (%)	CF
L50	2.26 ± 0.46^{ab}	4.06 ± 0.83^{c}	1.75 ± 0.09
L70	2.46 ± 0.45^{ab}	3.63 ± 1.28^{bc}	1.81 ± 0.09
L90	2.60 ± 0.89^{b}	3.84 ± 1.36^{bc}	1.80 ± 0.14
L110	2.47 ± 0.41^{ab}	3.78 ± 0.77^{bc}	1.88 ± 0.13
L130	2.05 ± 0.39^{ab}	2.75 ± 0.36^{a}	1.82 ± 0.12
L150	1.92 ± 0.38^{a}	2.47 ± 0.43^{a}	1.77 ± 0.09

Data represent mean ± S.E. (n = 3). Values in the same column with different superscripts are significantly different (P < 0.05).

Table 5
Proximate composition in whole body and muscle of juvenile *Nibea albiflora* fed different levels of lipid diets.

	Dietary lipid level					
	L50	L70	L90	L110	L130	L150
Whole body (%)						
Moisture	72.38 ± 0.01	71.77 ± 0.01	70.94 ± 0.02	70.89 ± 0.00	71.40 ± 0.00	70.26 ± 0.01
Crude protein	16.5 ± 1.49	16.59 ± 1.31	17.28 ± 0.63	17.08 ± 0.67	15.33 ± 1.32	16.16 ± 1.46
Crude lipid	5.99 ± 0.22^{a}	6.87 ± 0.65^{ab}	7.38 ± 0.90^{b}	7.70 ± 0.91^{b}	7.21 ± 0.28^{b}	8.06 ± 0.71^{b}
Ash	3.72 ± 0.41	3.69 ± 0.12	3.45 ± 0.30	3.73 ± 0.19	3.66 ± 0.36	3.84 ± 0.11
Muscle (%)						
Moisture	76.2 ± 1.23	76.8 ± 0.60	74.8 ± 3.20	75.7 ± 1.22	75.1 ± 1.02	73.6 ± 3.29
Crude protein	18.19 ± 2.22	17.54 ± 0.31	17.68 ± 3.23	18.87 ± 1.93	16.94 ± 1.22	19.72 ± 2.06
Crude lipid	3.94 ± 0.43^{a}	3.94 ± 0.17^{a}	5.16 ± 0.73^{ab}	5.24 ± 0.44^{ab}	5.20 ± 0.27^{ab}	5.67 ± 1.10^{b}
Ash	1.27 ± 0.01	1.31 ± 0.18	1.28 ± 0.14	1.28 ± 0.18	1.22 ± 0.03	1.40 ± 0.07

Data represent mean ± S.E. (n = 3). Values in the same row with different superscripts are significantly different (P < 0.05).

as Gulf corvina *C. othonopterus* (11.4%, Gonzalez-Felix et al., 2015) and Yellow croaker *Pseudosciaena crocea* (10.5%, Duan et al., 2001).

In fish, liver is considered a major fat and glycogen deposition site (Peres and Oliva-Teles, 1999; Chatzifotis et al., 2010). However, divergent effects of dietary lipids on hepatosomatic index (HSI) have been reported in previous studies. For instance, no differences in HSI due to changes in dietary lipid levels were found for Atlantic halibut (Martins et al., 2007; Helland and Grisdale-Helland, 1998),

Table 6
Plasma biochemical parameters of *Nibea albiflora* fed different levels of lipid diets.

	Dietary lipid level					
	L50	L70	L90	L110	L130	L150
TG(mmol L^{-1})	1.79 ± 0.36^{a}	1.95 ± 0.50^{a}	2.71 ± 1.88^{a}	2.12 ± 1.31^{a}	3.89 ± 1.58^{ab}	6.63 ± 3.19^{b}
CHOL(mmol L^{-1})	2.39 ± 0.15^{a}	3.99 ± 0.43^{b}	4.13 ± 0.52^{b}	4.59 ± 0.82^{b}	3.93 ± 0.29^{b}	4.96 ± 1.12^{b}
TP(g L^{-1})	27.2 ± 4.34	27.6 ± 2.5	29.1 ± 1.25	29.0 ± 0.95	25.3 ± 3.08	28.4 ± 2.38
GLU(mmol L^{-1})	4.07 ± 1.15^{a}	5.14 ± 1.35^{ab}	5.02 ± 0.94^{ab}	6.04 ± 2.03^{abc}	8.47 ± 0.68^{c}	7.44 ± 1.83^{bc}

Data represent mean ± S.E. ($n = 3$). Values in the same row with different superscripts are significantly different ($P < 0.05$).
TG, triglyceride, CHOL, cholesterol, TP, total protein, GLU, glucose.

Table 7
Fatty acid composition (mg g^{-1}) of liver tissue of juvenile *Nibea albiflora* fed different levels of lipid diets.

Lipid level	L50	L70	L90	L110	L130	L150
C14:0	1.78 ± 0.96	1.59 ± 0.11	1.67 ± 0.16	2.14 ± 0.05	2.02 ± 0.26	2.61 ± 0.86
C15:0	0.16 ± 0.08^{a}	0.19 ± 0.04^{ab}	0.15 ± 0.01^{a}	0.28 ± 0.03^{ab}	0.24 ± 0.06^{ab}	0.44 ± 0.12^{b}
C16:0	24.5 ± 2.98	23.9 ± 0.33	24.5 ± 1.17	26.4 ± 2.11	25.3 ± 0.35	23.0 ± 1.85
C16:1	12.4 ± 1.49	12.6 ± 0.80	12.9 ± 3.42	12.5 ± 0.54	12.6 ± 0.78	10.3 ± 1.07
C17:0	0.27 ± 0.09^{a}	0.30 ± 0.03^{a}	0.28 ± 0.03^{a}	0.40 ± 0.02^{ab}	0.31 ± 0.06^{a}	0.60 ± 0.1^{b}
C17:1	0.37 ± 0.01	0.41 ± 0.04	0.33 ± 0.05	0.41 ± 0.10	0.43 ± 0.04	0.49 ± 0.06
C18:0	9.97 ± 0.68	9.96 ± 0.38	10.7 ± 1.43	7.16 ± 1.09	8.49 ± 1.57	6.47 ± 1.85
C181n9	33.9 ± 4.42^{b}	31.8 ± 2.99^{ab}	33.6 ± 0.99^{b}	25.4 ± 0.76^{ab}	26.5 ± 2.35^{ab}	21.4 ± 4.48^{a}
C18:2n6	6.66 ± 2.38	6.19 ± 0.83	4.62 ± 0.43	6.66 ± 0.29	5.41 ± 0.87	6.94 ± 2.47
C20:0	0.22 ± 0.00	0.30 ± 0.00	0.28 ± 0.01	0.28 ± 0.13	0.36 ± 0.04	0.42 ± 0.03
C18:3n6	0.11 ± 0.05	0.11 ± 0.00	0.12 ± 0.03	0.10 ± 0.00	0.09 ± 0.02	0.10 ± 0.03
C18:3n3	0.88 ± 0.22	1.00 ± 0.16	0.80 ± 0.08	1.19 ± 0.01	1.08 ± 0.19	1.50 ± 0.44
C22:1n9	0.18 ± 0.01^{a}	0.16 ± 0.01^{a}	0.37 ± 0.14^{ab}	0.17 ± 0.06^{a}	0.59 ± 0.14^{b}	1.05 ± 0.03^{c}
C20:3n3	0.08 ± 0.02^{a}	0.10 ± 0.02^{a}	0.08 ± 0.01^{a}	0.12 ± 0.01^{ab}	0.13 ± 0.02^{ab}	0.17 ± 0.02^{b}
C20:4n6	0.32 ± 0.05^{ab}	0.33 ± 0.05^{ab}	0.29 ± 0.02^{a}	0.38 ± 0.00^{ab}	0.39 ± 0.02^{ab}	0.52 ± 0.12^{b}
C23:0	0.24 ± 0.05	0.22 ± 0.01	0.22 ± 0.02	0.18 ± 0.05	0.23 ± 0.00	0.21 ± 0.01
C22:2	0.18 ± 0.02^{a}	0.29 ± 0.06^{ab}	0.24 ± 0.02^{ab}	0.39 ± 0.02^{bc}	0.38 ± 0.05^{bc}	0.49 ± 0.05^{c}
C20:5n3 (EPA)	1.89 ± 0.08^{a}	2.79 ± 0.49^{ab}	2.56 ± 0.27^{a}	3.90 ± 0.13^{ab}	3.83 ± 0.37^{ab}	5.68 ± 1.64^{b}
DPA	0.30 ± 0.01^{a}	0.48 ± 0.10^{a}	0.33 ± 0.07^{a}	0.62 ± 0.00^{ab}	0.62 ± 0.05^{ab}	0.91 ± 0.20^{b}
C22:6n3 (DHA)	3.98 ± 0.82^{a}	6.25 ± 1.67^{a}	4.47 ± 1.24^{a}	9.05 ± 0.05^{ab}	8.89 ± 0.14^{ab}	13.7 ± 3.03^{b}
$\sum$SFA	37.3 ± 3.34	36.7 ± 1.27	37.8 ± 1.54	36.9 ± 0.86	37.1 ± 1.62	33.3 ± 3.11
$\sum$MUFA	48.4 ± 3.30^{b}	45.4 ± 2.09^{ab}	47.7 ± 2.83^{ab}	39.5 ± 1.21^{ab}	41.3 ± 0.14^{ab}	35.4 ± 5.91^{a}
$\sum$n-3	7.12 ± 0.15^{a}	10.62 ± 2.44^{a}	8.23 ± 1.67^{a}	14.89 ± 0.10^{ab}	14.56 ± 0.76^{ab}	21.92 ± 5.33^{b}
$\sum$n-3 HUFA	6.17 ± 0.75^{a}	9.52 ± 2.26^{a}	7.36 ± 1.58^{a}	13.57 ± 0.07^{ab}	13.35 ± 0.56^{ab}	20.25 ± 4.87^{b}

Data represent mean ± S.E. ($n = 3$). Values in the same row with different superscripts are significantly different ($P < 0.05$).
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA saturated fatty acids; MUFA mono-unsaturated fatty acids; HUFA highly-unsaturated fatty acids.

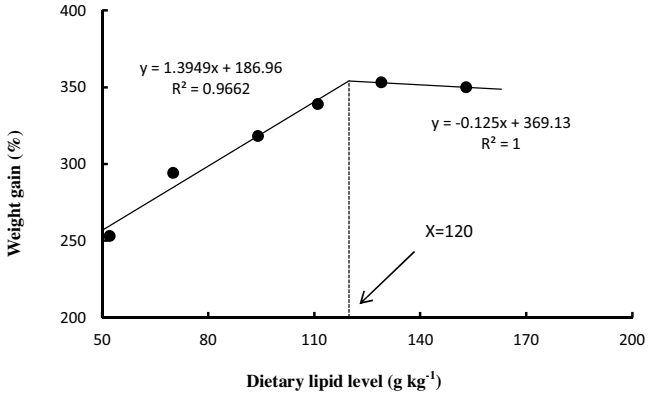

Fig. 1. The relationship between fish weight gain and dietary lipid level was assessed using the broken-line model, and the break point was obtained at 120 g kg^{-1} lipid level.

brown meager (Chatzifotis et al., 2006, 2010) and white seabream (Sá et al., 2006). On the contrary, other studies indicated that dietary lipid levels are positively (Cobia, Wang et al., 2005; Giant croaker, Han et al., 2014) or negatively (Grass carp, Du et al., 2005; European sea bass, Peres and Oliva-Teles, 1999) correlated with HSI. In this study, HSI of fish showed a decreasing trend with increasing dietary lipid level. Peres and Oliva-Teles (1999) suggested that HSI correlates with dietary carbohydrate level and hepatic glycogen amount. In this study, all diets contained the same protein and starchamounts, and differed only in lipid levels. Viscera somatic index (VSI) is an important trait directly affecting the yield of fish (Wang et al., 2005), and higher fat deposition in viscera can reduce the commercial value of fish product (Otwell and Rickards, 1981). As shown above, VSI of fish showed a decreasing trend, corresponding to HSI. However, these findings contrasted with data reported in other fish fed different lipid diets (Wang et al., 2005; Ghanawi et al., 2011). Condition factor (CF) is a crude measure of energy reserve levels (Goede and Barton, 1990) and fish health, and changes in CF may indicate variations in nutritional status of the fish (Chatzifotis et al., 2010). In this study, no significant differences were observed

in CF among all dietary treatments, corroborating previous reports (Ahmadi Fackjouri et al., 2011; Han et al., 2014).

The proximate composition of cultured fish is affected by endogenous and exogenous factors. Protein levels and ash amounts are mostly related to fish size (endogenous factors), while lipid levels are associated with exogenous factors such as diet (Chatzifotis et al., 2010). It was showed that whole body and muscle lipid contents increased with the increase of dietary lipid levels. Similar results were described for other species such as white seabass *Atractoscion nobilis* (Lopez et al., 2009), meagre *Argyrosomus regius* (Chatzifotis et al., 2010), grass carp *Ctenopharyngodon idella* (Jin et al., 2013) and giant croaker *Nibea japonica* (Han et al., 2014).

Fish react rapidly and sensitively to changes in dietary fatty acid composition, and dietary fatty acid pattern directly influences the tissue fatty acid profile (Mishra and Samantaray, 2004). As shown above, fatty acid profiles in the diets were well reflected by the liver fatty acid profiles in *N. albiflora*, in accordance with previous findings in *Atlantic halibut* (Martins et al., 2007) and rohu (Mishra and Samantaray, 2004). For marine fish species, essential fatty acid requirements are chiefly met by dietary n-3 highly-unsaturated fatty acid (HUFA), EPA and DHA (Sargent et al., 1999; Biswas et al., 2009). n-3 HUFA, rich in phospholipids, plays a key role in the maintenance of biomembrane structure, and its oxidation is restricted in fish tissues. On the contrary, the monoenes, more associated with neutral lipids, are readily oxidized for energy provision (Sargent et al., 1989; Mishra and Samantaray, 2004). In this study, total n-3 HUFA contents in liver, especially DHA and EPA amounts, were positively correlated with the dietary lipid levels; while, total mono-unsaturated fatty acid (MUFA) and saturated fatty acid (SFA) decreased with increasing dietary lipid levels. These results suggested that *N. albiflora* preferably utilizes MUFA and SFA as energy sources and preferentially retains HUFA, especially DHA and EPA, for physiological purposes (Arslan et al., 2013; Martins et al., 2007). The DHA/EPA ratio is important for growth performance of marine fish larvae and juveniles (Seoka et al., 2008), with values above 1.0 is appropriate for normal growth of Pacific bluefin tuna *Tunnus orientalis* (Biswas et al., 2009), in agreement with our data. The different dietary lipid levels can induce modifications in fatty acid composition and enzyme activity in intestinal brush border membranes of fish, affecting the membrane's physicochemical characteristics and intestinal digestive functions (Cahu et al., 2000; Gawlicka et al., 2002; Lopez et al., 2009). Further research is needed to determine the dietary fatty acid requirements for *N. albiflora*.

In this study, the plasma triglyceride and cholesterol contents of juvenile *N. albiflora* increased with the increasing dietary lipid level indicating a more active endogenous lipid transport, in response to the higher dietary lipid level (Du et al., 2005; Babin and Vernier, 1989), which is similar with previous study (Ding et al., 2010). Total plasma protein was a sensitive indicator of a dietary protein status (Pond et al., 1980). No significances were observed in plasma total protein contents among all treatments, the part of the reason maybe that all diets contained the same protein amounts in this study.

5. Conclusion

This is the first trial assessing the effect of dietary lipid level on growth performance, body composition, plasma biochemical parameters and liver fatty acids content in *N. albiflora*. Increasing dietary lipid level significantly enhanced weight gain, specific growth rate and feed utilization in *N. albiflora*. Our data suggest that a dietary lipid level of 120 g kg^{-1} might be optimal for *N. albiflora*, based on a broken-line model of percent weight gain.

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